In-Situ Formation and Alignment of Biological Supramolecular Assemblies in Microchannel Devices

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In Collaboration with (at UCSB)

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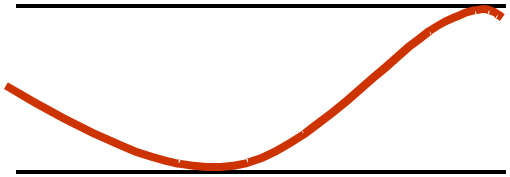
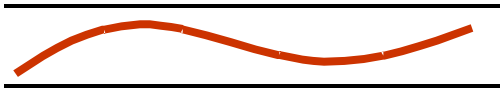
Alignment of self-assembled structures in microchannels

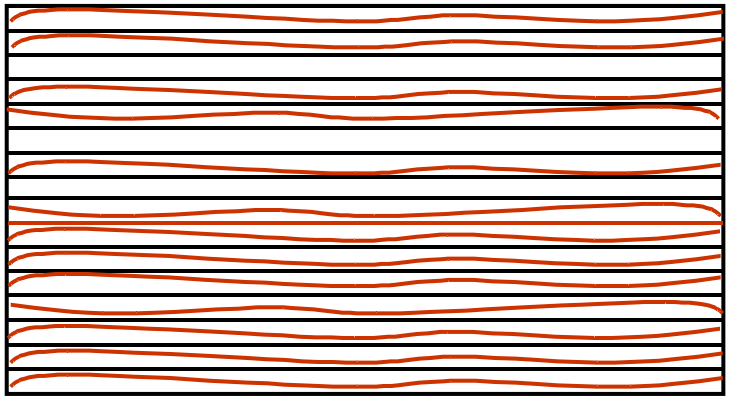
•Design a system to **geometrically confine** filamentous biomolecular self-assemblies.

•we must take into account the persistence length of the molecule/structure.





•Create a large **array** of confined molecules, to produce a highly aligned protein sample.

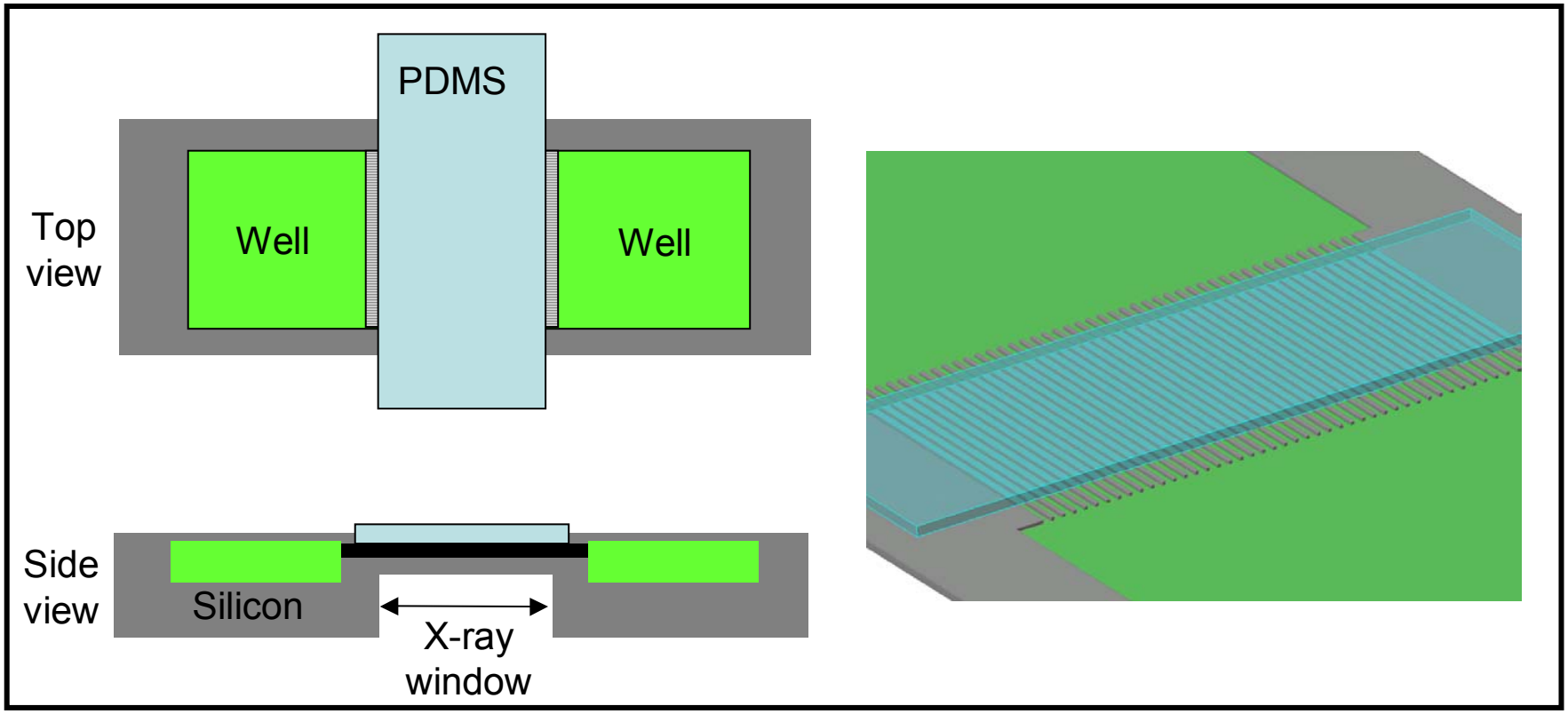


•Use **x-ray diffraction** to study small amounts of aligned protein samples

Other alignment methods – shearing, magnetic fields
larger amounts of protein

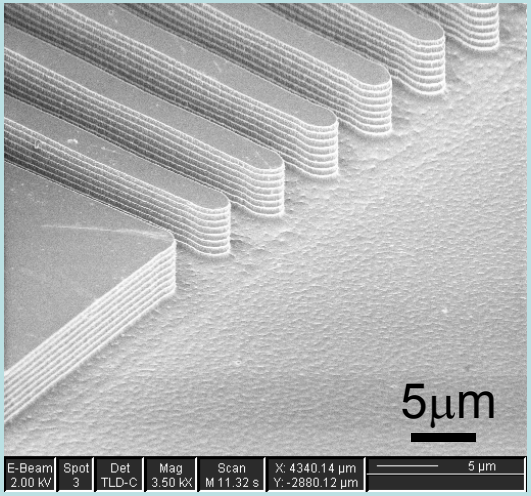
Microchannel system Design

- The micro channels are designed to be easily filled by a user by capillary flow
- The channels are coated with a PEG ‘comb’ to resist protein absorption.
- The wells are used to fill the channels with different protein solutions.
- Bundles self-assemble in the channels



Microfabrication of the microchannel

Silicon



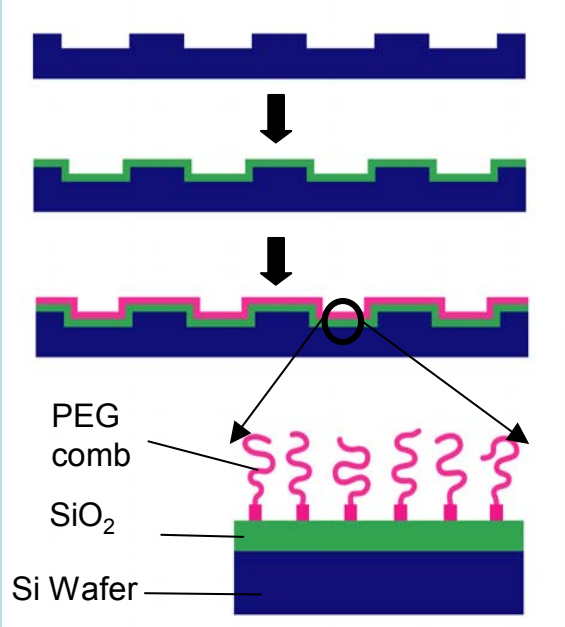
Standard BOSCH etch process

High aspect ratio features

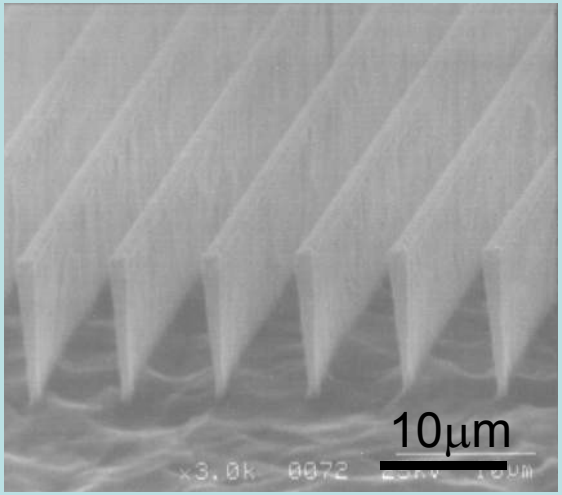
Channel structure etched into Silicon wafer

SiO_2 thermally grown on the surface

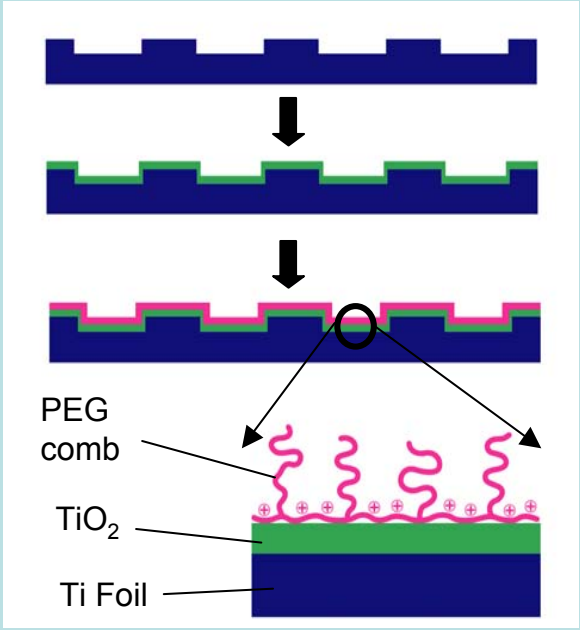
PEG-Silane absorbed to the surface



Titanium



Chlorine based TIDE etch.
High aspect ratio.
Smooth walls
robust material
Thin and strong



Channel structure etched into Titanium foil

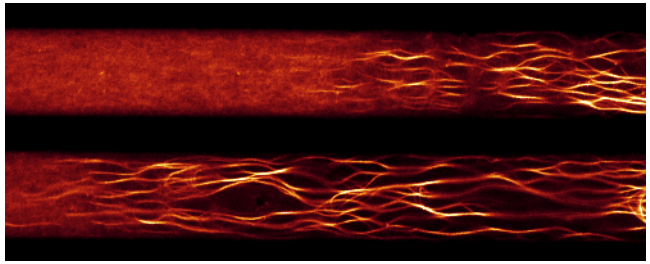
TiO_2 Sputtered on the surface

PLL-PEG absorbed to the surface

Filling the Microchannels with actin/α-actinin bundles

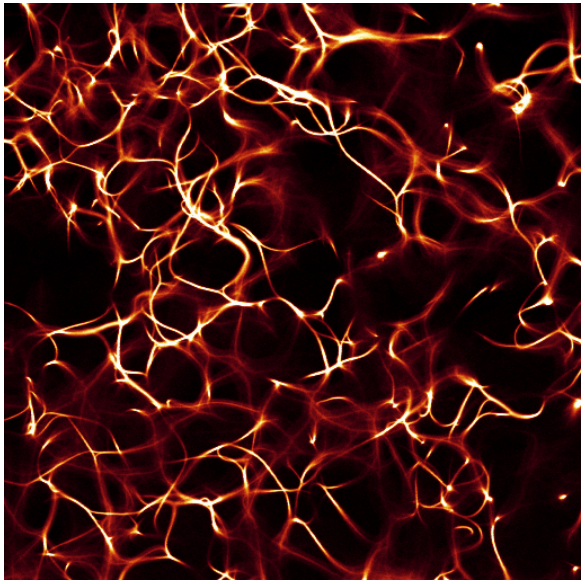
- G-Actin is polymerized *in-situ* in the presence of the linker molecule - Like behavior in the cell.
- Bundles grow along the channel, aligned by confinement.
- The wider the channel, the less alignment is observed.

α-actinin conc. →

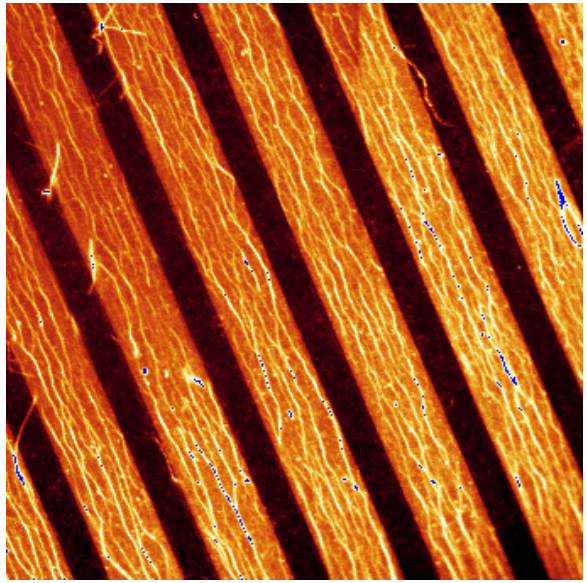


Bundle growth in microchannels

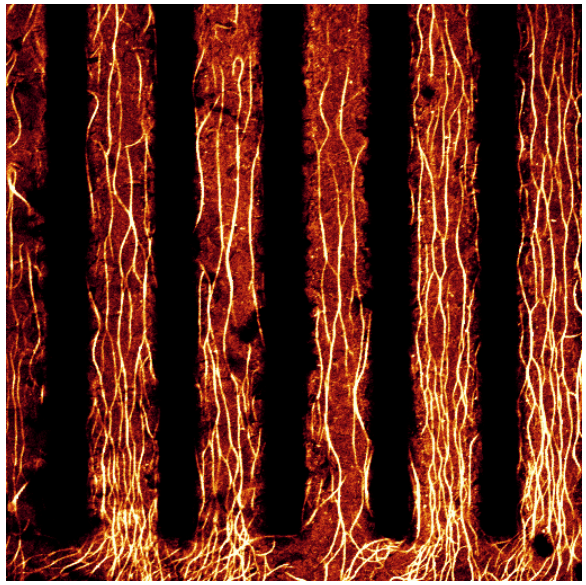
Confocal fluorescence microscope images



No channels

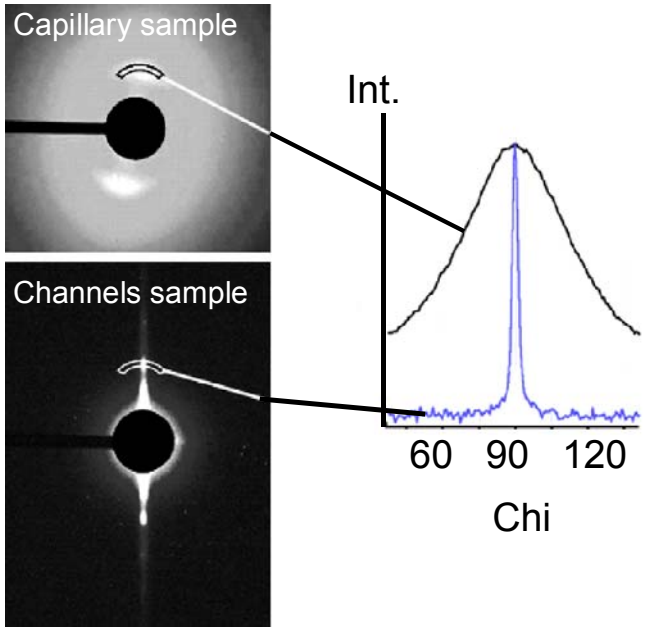


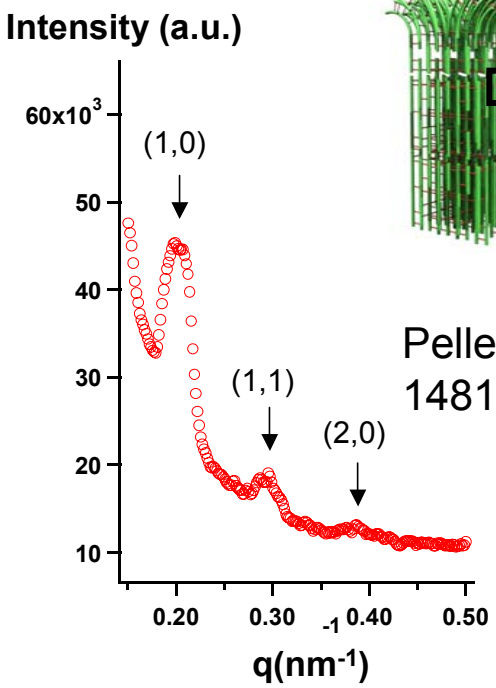
10μm Ti channels

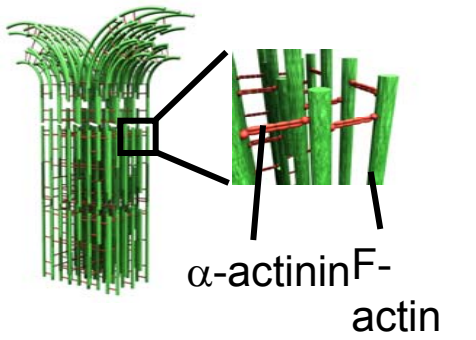


20μm Ti channels

Microchannel Device X-ray Scattering Results







Pelletier, et al, **PRL** 91, 148102 (2003)

•Samples prepared in the channels system give **highly aligned** results with **very little material** compared to a typical capillary sample.

•For our **actin/α-actinin system** we observe the expected peaks for a quasi-square structure.

N. Bouxsein, L.S. **Hirst**, Z. Abu Samah, Y. Li and C.R. Safinya *APL* **85**, 5777 (2004)

L.S. Hirst et al. **Langmuir 21**, 3910 (2005)

X-ray & Neutron Requirements

- Micro- to nano-sized beams
- In-situ multi-probes at multiple lengthscales
- Parallel automated sample handling and data acquisition
- Issues with signal and radiation damage